

SUBSTITUTE SPECIFICATION – CLEAN VERSION FOR PROSECUTION

CLAIMS

1. A polynucleotide encoding a cytolethal distending toxin, which is any one of:
 - (a) a polynucleotide encoding a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 2 to 4;
 - (b) a polynucleotide comprising any one of the nucleotide sequences of position 1 to 777, 802 to 1605, and 1615 to 2187 in the nucleotide sequence of SEQ ID NO: 1;
 - (c) a polynucleotide encoding a polypeptide comprising an amino acid sequence with a substitution, deletion, addition, and/or insertion of one or more amino acids in any one of the amino acid sequences of SEQ ID NOs: 2 to 4;
 - (d) a polynucleotide that hybridizes under a stringent condition to DNA comprising any one of the nucleotide sequences of position 1 to 777, 802 to 1605, and 1615 to 2187 in the nucleotide sequence of SEQ ID NO: 1;
 - (e) a polynucleotide encoding a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 52 to 54;
 - (f) a polynucleotide comprising any one of the nucleotide sequences of position 1 to 702, 778 to 1629, and 1632 to 2177 in the nucleotide sequence of SEQ ID NO: 51;
 - (g) a polynucleotide encoding a polypeptide comprising an amino acid sequence with a substitution, deletion, addition, and/or insertion of one or more amino acids in the amino acid sequence of any one of SEQ ID NOs: 52 to 54; and
 - (h) a polynucleotide that hybridizes under a stringent condition to DNA comprising any one of the nucleotide sequences of position 1 to 702, 778 to 1629, and 1632 to 2177 in the nucleotide sequence of SEQ ID NO: 51.
2. A vector comprising the polynucleotide of claim 1.
3. A host cell containing the polynucleotide of claim 1 or the vector of claim 2.
4. A polypeptide encoded by the polynucleotide of claim 1.
5. A method for producing the polypeptide of claim 4, which comprises the step of culturing the host cell of claim 3 and collecting the produced polypeptide from the host cell or the culture supernatant.
6. An antibody that binds to the polypeptide of claim 4.

SUBSTITUTE SPECIFICATION – CLEAN VERSION FOR PROSECUTION

7. A method for detecting the presence of *Campylobacter* bacteria in a test sample, which comprises the steps of:

(a) conducting a nucleic acid amplification reaction on the test sample using a common primer pair that can amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter* bacteria; and

(b) determining the presence of *Campylobacter* based on the presence or molecular weight of an amplified fragment from the genomic DNA encoding the cytolethal distending toxin of the *Campylobacter* bacterium.

8. The method of claim 7, wherein the *Campylobacter* bacterium is *Campylobacter coli*, *Campylobacter jejuni*, and/or *Campylobacter fetus*.

9. A method for detecting the presence of *Campylobacter coli*, *Campylobacter jejuni*, and *Campylobacter fetus* in a test sample, which comprises the steps of:

(a) conducting a nucleic acid amplification reaction on the test sample using a mixture of primer pairs specific to each of genomic DNAs encoding the cytolethal distending toxins of these bacteria; and

(b) determining the presence of the bacteria based on the presence or molecular weight of amplified fragments from the genomic DNAs encoding the cytolethal distending toxins of the bacteria.

10. A method for detecting the presence of *Campylobacter coli*, *Campylobacter jejuni*, and *Campylobacter fetus* in a test sample, which comprises the steps of:

(a) conducting a nucleic acid amplification reaction on the test sample using a common primer pair that can amplify genomic DNAs encoding the cytolethal distending toxins of these bacteria;

(b) conducting a nucleic acid amplification reaction on the test sample or with the genomic DNA amplified in step (a) as a template using a mixture of primer pairs specific to each of genomic DNAs encoding the cytolethal distending toxins of the bacteria; and

(c) determining the presence of the bacteria based on the presence or molecular weight of amplified fragments from the genomic DNAs encoding the cytolethal distending toxins of the bacteria.

11. The method of claim 7, 8, or 11, wherein the common primer pair is any one of a primer pair comprising the sequences of SEQ ID NOs: 64 and 65, a primer pair selected from SEQ ID NOs: 7 to 10 and 47 to 50, a primer pair comprising the sequences of SEQ ID NOs: 66

SUBSTITUTE SPECIFICATION – CLEAN VERSION FOR PROSECUTION

and 67, and a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

12. The method of claim 9 or 10, wherein the method uses (a) to (c) as the mixture of specific primer pairs:

(a) a primer pair comprising SEQ ID NOs: 70 and 71 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(b) a primer pair comprising SEQ ID NOs: 68 and 69 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair; and

(c) a primer pair comprising SEQ ID NOs: 72 and 73 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

13. The method of claim 9 or 10, wherein the method uses (a) to (c) as the mixture of specific primer pairs:

(a) a primer pair selected from SEQ ID NOs: 13, 14, and 28 to 36 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(b) a primer pair selected from SEQ ID NOs: 11, 12, and 17 to 27 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair; and

(c) a primer pair selected from SEQ ID NOs: 15, 16, and 37 to 46 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

14. The method of claim 9 or 10, wherein the method uses (a) to (c) as the mixture of specific primer pairs:

(a) a primer pair comprising SEQ ID NOs: 76 and 77 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(b) a primer pair comprising SEQ ID NOs: 74 and 75 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair; and

(c) a primer pair comprising SEQ ID NOs: 78 and 79 to amplify a genomic DNA

SUBSTITUTE SPECIFICATION – CLEAN VERSION FOR PROSECUTION

encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified said the primer pair.

15. A method for detecting the presence of *Campylobacter coli*, *Campylobacter jejuni*, and *Campylobacter fetus* in a test sample, which comprises the steps of:

(a) conducting a nucleic acid amplification reaction on the test sample using a common primer pair that can amplify genomic DNAs encoding *cdtB* subunits of the cytolethal distending toxins of these bacteria;

(b) digesting the genomic DNA amplified in step (a) with a restriction enzyme; and

(c) determining the presence of the bacteria based on the molecular weight of a DNA fragment resulting from the digestion.

16. The method of claim 15, wherein the restriction enzyme is selected from the group consisting of: *Sau3AI*, *DsaI*, *MboI*, *RsaI*, *EcoRI*, *HinfI*, *NdeI*, *PstI*, *XbaI*, and *XhoII*.

17. The method of claim 15, wherein the common primer pair is a primer pair selected from SEQ ID NOs: 7 to 10 and 47 to 50 or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

18. A kit used in the method of claim 7 or 8, which comprises an instruction manual and a common primer pair that can amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter* bacteria.

19. The kit of claim 18, wherein the common primer pair is any one of a primer pair comprising the sequences of SEQ ID NOs: 64 and 65, a primer pair selected from SEQ ID NOs: 7 to 10 and 47 to 50, a primer pair comprising the sequences of SEQ ID NOs: 66 and 67, and a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

20. A kit used in the method of claim 9, which comprises an instruction manual and a mixture of primer pairs specific to each of genomic DNAs encoding the cytolethal distending toxins of *Campylobacter coli*, *Campylobacter jejuni*, and *Campylobacter fetus*.

21. The kit of claim 20, wherein the mixture of specific primer pairs is as follows:

(a) a primer pair comprising SEQ ID NOs: 70 and 71 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

SUBSTITUTE SPECIFICATION – CLEAN VERSION FOR PROSECUTION

(b) a primer pair comprising SEQ ID NOs: 68 and 69 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair; and

(c) a primer pair comprising SEQ ID NOs: 72 and 73 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair:

22. The kit of claim 20, wherein the mixture of specific primer pairs is as follows:

(a) a primer pair selected from SEQ ID NOs: 13, 14, and 28 to 36 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(b) a primer pair selected from SEQ ID NOs: 11, 12, and 17 to 27 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(c) a primer pair selected from SEQ ID NOs: 15, 16, and 37 to 46 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

23. The kit of claim 20, wherein the mixture of specific primer pairs is as follows:

(a) a primer pair comprising SEQ ID NOs: 76 and 77 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(b) a primer pair comprising SEQ ID NOs: 74 and 75 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair; and

(c) a primer pair comprising SEQ ID NOs: 78 and 79 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

24. A kit used in the method of claim 20, which comprises an instruction manual and the following (a) and/or (b):

(a) a mixture of primer pairs specific to each of genomic DNAs encoding the cytolethal distending toxins of *Campylobacter coli*, *Campylobacter jejuni*, and *Campylobacter fetus*; and

(b) a common primer pair that can amplify genomic DNAs encoding the cytolethal distending toxins of *Campylobacter coli*, *Campylobacter jejuni*, and *Campylobacter fetus*.

SUBSTITUTE SPECIFICATION – CLEAN VERSION FOR PROSECUTION

25. The kit of claim 24, wherein the mixture of specific primer pairs is as follows:

(a) a primer pair comprising SEQ ID NOs: 70 and 71 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(b) a primer pair comprising SEQ ID NOs: 68 and 69 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(c) a primer pair comprising SEQ ID NOs: 72 and 73 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

26. The kit of claim 24, wherein the mixture of specific primer pairs is as follows:

(a) a primer pair selected from SEQ ID NOs: 13, 14, and 28 to 36 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(b) a primer pair selected from SEQ ID NOs: 11, 12, and 17 to 27 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair; and

(c) a primer pair selected from SEQ ID NOs: 15, 16, and 37 to 46 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

27. The kit of claim 24, wherein the mixture of specific primer pairs is as follows:

(a) a primer pair comprising SEQ ID NOs: 76 and 77 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter coli*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair;

(b) a primer pair comprising SEQ ID NOs: 74 and 75 to amplify genomic DNA encoding the cytolethal distending toxin of *Campylobacter jejuni*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair; and

(c) a primer pair comprising SEQ ID NOs: 78 and 79 to amplify a genomic DNA encoding the cytolethal distending toxin of *Campylobacter fetus*, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

28. The kit of any one of claims 24 to 27, wherein the common primer pair is selected from a primer pair of the sequences of SEQ ID NOs: 65 and 64, a primer pair selected from SEQ

SUBSTITUTE SPECIFICATION – CLEAN VERSION FOR PROSECUTION

ID NOs: 7 to 10 and 47 to 50, and a primer pair of the sequences of SEQ ID NOs: 66 and 67, or is a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.

29. A kit used in the method of claim 15, which comprises an instruction manual and a common primer pair that can amplify genomic DNAs encoding the cdtB subunit of the cytolethal distending toxins of *Campylobacter coli*, *Campylobacter jejuni*, and *Campylobacter fetus*.

30. The kit of claim 29, wherein the common primer pair is a primer pair selected from SEQ ID NOs: 7 to 10 and 47 to 50, or a primer pair that can amplify the same genomic DNA region as amplified with said primer pair.